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ABSTRACT

The Ediacaran-Cambrian cloudinomorphs, which include *Cloudina*, are the first putative skeletal metazoans. They have a benthic ecology and tubular, organic or biomineralized stacked funnel morphologies, but an unresolved phylogenetic affinity. Rare dichotomous branching has been described in *Cloudina,* but here we demonstrate the presence of multiple (polytomous), dichotomous branching in cloudinomorphs from a microbial mat-community from the Nama Group, Namibia, as revealed by 3D models created from serial sections. Branches share an open, central cavity, and branching is achieved via external budding. These cloudinomorphs show attachment and mutual cementation to each other, and also to *Namacalathus,* via extratubular skeletal structures to potentially form a horizontal framework. Polytomous branching excludes a bilaterian affinity as proposed for other cloudinomorphs. This raises the possibility that the Ediacaran tubular, funnel morphology is convergent, and that cloudinomorphs may, in fact, represent taxa of diverse affinity.

INTRODUCTION

Significant evolutionary innovations in metazoans such as biomineralization, reef-building, and motile behavior appear during the terminal Ediacaran. The most studied Ediacaran skeletal taxa is *Cloudina* (ca. 550-540 Ma), due to its widespread distribution and potential index fossil status (Grant, 1990).

Cloudina and other similar tubular skeletal and organic taxa (informally known as 'cloudinids' and 'cloudinomorphs') have a sinuous, tube-like morphology formed of eccentrically stacked funnels that lack transverse cross-walls but which can flare to form outer wall flanges (Germs, 1972; Hua et al., 2005; Selly et al., 2020). To date, at least seven genera have been attributed to cloudinomorphs (Yang et al., 2020), and as a group they are transitional across the Ediacaran-Cambrian boundary. Cloudinomorphs can have a closed or open base, a smooth inner wall, and an aperture (Germs, 1972; Cai et al., 2011; Schiffbauer et al., 2020). The size of *Cloudina* is highly variable globally, cited as ranging from 0.3 to 6.5 mm wide and 1.5 to 150 mm in length; dimensions may be determined by environmental as well as systematic factors (Wood et al., 2017). *Cloudina* communities occupied a range of different carbonate substrates including microbial mats, thrombolites, and reefs (Cai et al., 2014; Penny et al, 2014; Becker-Kerber et al., 2017; Wood et al., 2017, Álvaro et al., 2019, but see Mehra and Maloof, 2018). Dichotomous branching has been observed in a few cases in *Cloudina* (Hua et al., 2005; Penny et al, 2014) and also budding of daughter tubes between two adjacent tubes (Cortijo et al., 2010), both suggestive of asexual reproduction. Evidence for mutual attachment and cementation of *Cloudina* individuals has been noted in the Nama Group, Namibia, where two forms of attachment are documented: apical terminations serving as attachment sites and 'meniscus cements' between adjacent individuals (Penny et al., 2014).

The affinity of cloudinomorphs is problematic, with the group variously attributed to calcareous algae, cnidarians or annelids (Terleev et al., 2004, e.g. Vinn and Zaton, 2012;

Schiffbauer et al., 2020). A cnidarian affinity is supported by the deep-seated division of *Cloudina* tubes within the parent tube (Hua et al., 2005; Cortijo et al., 2014). However, the general nested funnel morphology and skeletal ultrastructure of some cloudinomorphs are similar to modern annelids (Vinn and Zaton, 2012; Yang et al., 2020), and preservation of a central, tubular, pyritized structure in a cloudinomorph inferred to be a gut would support a stem-annelid, affinity (Schiffbauer et al., 2020). Indeed many skeletal features of cloudinomorphs are in fact present in diverse invertebrate groups.

Here we describe the first recorded instance of multiple (polytomous) branching, and the form of attachment sites, in cloudinomorphs. This informs the debate as to the potential affinities of this global and transitional Ediacaran-Cambrian group.

GEOLOGICAL SETTING

The Nama Group (ca. 550–541 Ma) is a fossiliferous Ediacaran-Cambrian sedimentary ramp succession deposited in two sub-basins (Germs, 1983, and see Supplementary Material and Fig. DR1 in the Data Repository), consisting of the Kuibis Subgroup overlain by the Schwarzrand (Germs, 1983; Saylor et al., 1995). Ash bed dates and inferred sedimentation rates suggest the basal Nama unconformity is ca.550–553 Ma (Saylor et al., 1998) and the top of the Schwarzrand Subgroup is younger than at least 539.64 ± 0.19 Ma (Linnemann et al., 2019).

MATERIAL AND METHODS

Samples from *in-situ* bedding planes were collected from near the top of the Upper Omkyk Member of the Kuibis Subgroup, at Omkyk Farm and Driedoornvlagte in the Zaris Subbasin (Table DR1; Fig. DR1). An ash bed from the overlying Hoogland Member is dated at 547.32 ± 0.65 Ma (Grotzinger et al., 1995; Schmitz, 2012). Driedoornvlagte is a shallow

reef complex that formed in a mid-ramp setting that contains abundant skeletal taxa, *Cloudina, Namacalathus,* and *Namapoikia*. At Omkyk Farm, strata record deposition at an inner ramp position close to the Osis Arch, into increasingly shallow, very low-energy, lagoonal settings containing *Cloudina* and *Namacalathus* (Wood et al., 2015).

Cloudinomorph specimens were serially ground in increments of either 10 μm (Figs. DR2 and 3), or 25 μm (Fig. DR4), using a Buehler Petrothin thin sectioning system and imaged using a binocular microscope. Processed images were imported as a virtual stack into Avizo 9 software to create 3D models composed of between 50-123 2D images and smoothed to reduce noise. Cloudinomorph walls and the open cavity were manually segmented separately (Fig. DR5). Highly polished thin sections were imaged by standard petrographic and cathodoluminesence microscopy, and ImageJ software was used for all measurements. See the GSA Data Repository[1] for further details of localities and methods.

RESULTS

Microbial mat metazoan communities

Bedding planes of flaggy, wackestone to packstone grading into friable, dolomitized wackestone to mudstone from Omkyk Farm, 12-30 mm thick, show abundant aggregations of sinuous, straight, and apparently branching cloudinomorphs and *Namacalathus* associated with inferred microbial mats (Figs. 1A and B; Fig. 2). Inferred microbial mats are dark or covered in iron oxides and have irregular or patchy edges distinct from the yellow, micritic mudstone with skeletal bioclasts. Mats are usually <1 mm thick and undulating, and occur parallel to bedding at a spacing of 2-5 mm.

Tubular fossils from Omkyk Farm show a distinct annulated outer wall structure (Fig. 1C) but recrystallization obscures any internal stacked, funnel organization. They are therefore

attributed to cloudinomorphs. Material from Driedoornvlagte can be attributed to *Cloudina* (Grant, 1990).

Cloudinomorph individuals range from 1.2-21.7 mm in length, and 0.1-2.3 mm in width (n=604) as measured on bedding planes, and are inferred to be attached to mats, as they are most abundant within inferred mats and some individuals appear to radiate from them (Fig. 1A). *In-situ Namacalathus* are present as clusters of small individuals within the mats (Fig. 1A), and as larger individuals outside the mats, which show close aggregation and radiate from a smaller area of attachment with deformation between individuals (Fig. 1B).

Cloudinomorph growth patterns

The diameter of cloudinomorphs measured from bedding surfaces shows an initial fast rate of inflation within the first 1 mm of extensional growth but remains constant thereafter (Fig. 1D). While bedding plane surfaces show examples of potential attachment sites and/or branching cloudinomorphs (Fig. 2), evidence for bifurcating tubes can only be proven by the presence of a shared cavity.

Polished surfaces reveal that cloudinomorph tubes are infilled first by a cloudy, isopachous cement (21-587 μm thick; mean = 110 μm; Fig. DR7), then remaining internal space by sparry calcite (Figs. DR2 and DR4). Cathodoluminescence reveals an earlier, thin (up to 200 μm) generation of acicular cement (Fig. DR6C). The isopachous cement is either patchy or zoned, and sparry calcite cements distinctly zoned (Figs. 3G, H, J). The presence of a shared cavity is evidenced by the absence of a wall or any associated skeletal breakage that might imply post-depositional compaction, and also that the isopachous cement crust forms a continuous crust connecting both tubes, as well as continuous sparry calcite cement infill (Figs. 3G, H).

One 3D model of the cloudinomorph outer wall, highlighted by the micritic envelope or the outer edge of the cavity lining isopachous cement, confirms the presence of multiple branches with differing orientations along one parent tube (Figs. 3A, B; Figs. DR2). These diverge from the parental tube at angles of 35 to 299° (Table DR2; Fig. DR8). The plunge angle of the branches and attachment site, are consistently shallow, from 1.4 to 7.1°. Models of the cavity as defined by the extent of sparry calcite confirms this multiple branching structure (Figs. 3C, D). The diameter of cloudinomorph branches measured directly from the 3D models also shows an initial fast rate of inflation within the first 1 mm of growth but constant diameter thereafter (Fig. 1D).

Branches grow from the outer wall of the parent tube, i.e. with no intra-calar bifurcation (Figs. 3A-D). Models also reveal attachment sites of small cloudinomorph individuals, as indicated by the presence of the parental tube outer wall and no shared cavity (Figs. 3A, B; Fig. DR2D, G). The attached tube is often initially more narrow than the width of the tube to which it attaches (Figs. 3A, B), but the tube inflates also rapidly to form a cavity (Fig. 1D). Individuals can therefore be reconstructed with both multiple branches and attachment sites (Fig. 3E).

Cloudinomorph attachment structures

Cementation can present as one tube attached either at the base (attachment) or along the length of the shell (mutual cementation). On the bedding surface, potential cemented/attached tubes are indicated by a gap between apparently branching tubes (Fig. 2E). This is inferred to represent either the isopachous cement crust or sediment infill between the parent and inflating daughter tube, or two attached tubes which are differently orientated in 3D relative to one another.

147 Mutual cementation is also observed at both Omkyk Farm and Driedoornvlagte between
148 adjacent or near parallel tubes in close promimity (< 0.3 mm) (Fig. 4A). At Driedoornvlagte,
149 *Cloudina* walls are composed of brown, inclusion-rich dolomite and dolomitized extra-
150 tubular structures, with geopetal dolomitized micrite present within the tube (Fig. 4D). Under
151 cathodoluminescence, *Cloudina* walls show bright luminescence and neomorphosed calcite
152 with the same bright luminescence is also present between the adjacent tubes suggesting that
153 the *Cloudina* walls and the extra-tubular structure share the same diagenetic signature (Fig.
154 4E). This structure predates the formation of a further distinctive, slightly bluish, dull
155 luminescent neomorphosed calcite with patchy areas of brighter luminescence, that forms
156 both adjacent to *Cloudina* walls and within the inner tube adjacent to the contact structure
157 (Fig. 4E). An inclusion rich, non-luminescent sparry calcitic cement and a later clear sparry
158 calcite postdate these cements (Fig. 4E).
159 Similar mutual cementation is also noted between cloudinomorphs and the outer wall of a
160 fragment of *Namacalathus* (Fig. 4B; Fig. DR4), reaching 0.4 mm thickness. Attachment is
161 further confirmed via serial sectioning and 3D modeling (Fig. 4C).
162

163 **DISCUSSION**

164 The low energy, inner ramp environment of the Upper Omkyk Member at Omkyk Farm
165 has allowed for the preservation of an inferred microbial mat community of delicate, in-situ
166 branching or cemented cloudinomorphs and *Namacalathus.*
167 The presence of a shared cavity in these cloudinomorphs shows that the parent and
168 daughter branches shared the same living space. The continuation of early cement between
169 the parent and daughter tube and the lack of evidence for breakage of the tube walls suggest
170 this is a growth feature. This style of branching is unlike that previously recorded where the
171 tube splits into two half cylinders within the parent tube, i.e. intra-parietal budding, or

dichotomous branching (Hua et al., 2005; Cortijo et al., 2010). We see no evidence of a dividing wall along the midline within the parent tube, suggesting a different form of reproduction, here conforming to external budding.

We find multiple examples of apical and longitudinal attachment accompanied by a neomorphosed calcite extratubular structure, which aided mutual cloudinomorph attachment. We further show the occurrence of attachment between cloudinomorph and *Namacalathus*. These structures pre-date all inorganic cements, including pseudomorphed aragonitic cement botryoids where present (Penny et al., 2014). Given that these structures are only found associated with cloudinomorphs, and also have multiple positions relative to the tubular growth form, it is likely that they have a biological origin. These structures may have formed by extra-tubular secretion, perhaps via extracellular polymeric substances (EPS) or an organic template, which became calcified. The close proximity of individuals appears to have encouraged mutual cementation allowing for the formation of a horizontal, multi-component, rigid structure associated with microbial mat surfaces (Fig. 4F). This confirms that the ability to gain secure, mutual, attachment was present by the terminal Ediacaran.

The observation of multiple branching in cloudinomorphs suggests a non-bilaterian affinity for these representatives. Modern cnidarians, in particular hydrozoans, show various forms of asexual reproduction, including longitudinal fission and external budding, rapid daughter branch diameter increase after branching, similar diameters of parent and daughter tubes, and multiple branching (Vinn and Zaton, 2012; Han et al., 2017; Yang et al., 2020). Such a non-bilaterian affinity would be counter to that implied by the presence of an inferred gut in cloudinomorphs from Nevada, USA (Schiffbauer et al., 2020), as well as the organized, lamellar microstructure of organic-walled cloudinomorphs from Mongolia (Yang et al., 2020). This apparent contradiction therefore raises the possibility that the common

Ediacaran-Cambrian tubular and stacked, funnel morphology is convergent, and that cloudinomorphs may, in fact, represent taxa of diverse affinity.

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FIGURE CAPTIONS

Figure 1: Bedding surfaces from Upper Omkyk Member, Omkyk Farm, Namibia. A: *In-situ* cloudinomorphs and *Namacalathus* (black arrows) on two generations of microbial mat surfaces (1 and 2) with projecting cloudinomorphs (red arrows), and bioclasts in inter-mat mudstone (3). B: Cluster of large *Namacalathus* in inter-mat area (arrow). C: Cloudinomorph with annulated morphology (funnels arrowed). D: Width of cloudinomorph tubes measured from 2D bedding planes and multiple branching 3D model.

Figure 2: Cloudinomorphs with multiple attachment sites and branches. A, C, E: Bedding plane images; B, D, F: Trace through inferred central axes of tubes. E, multiple attachment

293 sites with isopachous cements or sediment infill defining the contact between tubes (single
294 arrows) and inner tube wall (double arrow).
295
296 Figure 3: Multiple branching cloudinomorph with the parent tube (1, white arrow), branching
297 daughter tube (2; shared cavities, orange arrow), indirect evidence of branching where the
298 cavity does not extend to the full tube (green arrow), and attachment sites (3, red arrow). A:
299 Upper, plan view of 3D model (from bedding surface) of outer tube with 5% smoothing. B:
300 Lower, rear view of same model. C: Upper, plan view of 3D model (from bedding surface) of
301 open cavity, D: Lower, plan view of same model. E: Reconstruction of multiple branching
302 cloudinomorph (Artist: Astrid Robertsson). F: PPL photomicrograph and G:
303 Cathodoluminescent photomicrograph of branching cloudinomorph, showing continuity of
304 isopachous (I) and sparry calcite (S) cements in parent and daughter tube. H: Inset of G,
305 showing continuity of isopachous cement between the two tubes (blue arrows) and later
306 sparry cement infilling both (orange arrows). I: PPL photomicrograph and J:
307 Cathodoluminescent photomicrograph of cloudinomorph tube where arrows mark successive
308 funnels, with isopachous (I) and sparry calcite (S) cement infill.
309
310 Figure 4: Cloudinomorph attachment. A: Plan view of attached parallel cloudinomorph tubes
311 (white arrow) with stacked funnel morphology (red arrows indicate funnels). B: Plan view of
312 extra-tubular structure between cloudinomorph tube and *Namacalathus* (white arrow). C: 3D
313 model showing cementation between *Namacalathus* and a cloudinomorph (white arrow), blue
314 arrow indicates way up. D: PPL photomicrograph of two *Cloudina* tubes from
315 Driedoornvlagte, with inclusion rich extra tubular structure (ETS) between the tubes.
316 Geopetal dolomitic geopetal micrite (DM), and calcitic spar infill (CS). E:
317 Cathodoluminescent image of same area as D, showing two generations within the ETS,

bright luminescence (ETS^1) then dull luminescence with patches of bright luminescence (ETS^2). Both pre-date the inclusion-rich cement (IC) and latest sparry cements (CS). F: Reconstruction of inferred microbial mat-community with in-situ skeletal metazoans (1) Microbial mat; (2) *Namacalathus* cluster; (3) Isolated *Namacalathus* outside microbial mat; (4) Branching cloudinomorph with horizontal habit; (5) Multiple attachment sites of cloudinomorph; (6) Mutual attachment of cloudinomorph by extra-tubular structures; (7) Attachment between cloudinomorph and *Namacalathus*. (Artist: Astrid Robertsson).